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N-terminal Sequence Analysis of Endoproteinase Lys-C Peptide Map Using Routine 3.5 Method on the HP G1005A N-terminal Protein Sequencing System

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HP G1005A
N-terminal Protein Sequencing System

AN 96-1

Application Note

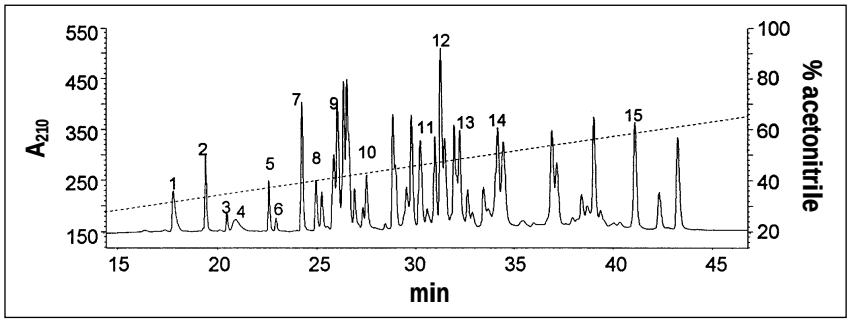

Figure 1. Reverse-phase HPLC fractionation of Lys-C digest of human apo-transferrin (200 pmol).

Abstract

This Application Note describes the N-terminal Sequence Analysis of peptide recovered from HPLC fractionated endoproteinase Lys-C digest.

Keywords

Sequence, peptide, G1005A

Introduction

N-terminal sequence analysis of peptides derived from proteolytic digests is routinely performed for protein identification, validation, and for the design of oligonucleotide probes. Endoproteinase Lys-C preferentially cleaves at the carboxy terminus of lysine residues and is often used for the digestion of proteins separated by SDS polyacrylamide gel electrophoresis (PAGE).

Methods

One nmole of human apo-transferrin (Sigma) was dissolved in 25 µl of buffer containing 8M urea, 100mM Tris.HCl (pH 9.0) and 10 mM DTT. The sample was incubated for 15 min at 60°C, resulting in sample denaturation and disulfide bond reduction. The volume was increased to 100 µl with 100 mM Tris.HCl (pH 9.0) and Endoproteinase Lys-C (Wako Chemicals USA, VA) was added to a ratio of 1:20 enzyme:substrate. The sample was digested at room temperature for 16 hours.

The HPLC fractionation of the digest (20 µl injection, 200 pmol) was performed on an HP 1090A HPLC fitted with a Vydac C-18 reverse-phase 2.1 mm × 25 cm column. Separation was achieved with a linear acetonitrile gradient (95% solvent A [0.1% TFA] to 100% solvent B [70% acetonitrile, 0.085% TFA] over 60 min; flowrate of 0.2 mL/min, 40°C column temperature and monitored at 210 nm.

Peptide fractions were diluted approximately 10-fold in 1 mL of 2% aqueous TFA and directly loaded on reverse-phase (RP) sequencing columns (HP G1073A), previously subjected to automated column prep using “Column prep 3.1/3.5” method. Each of the peptides was successfully sequenced through the C-terminus lysine residue using the “Routine 3.5” sequencing method.

Results and Discussion

The peptide map generated by HPLC fractionation of the Lys-C digestion of human apo-transferrin is shown in Figure 1. The chromatography gradient is indicated by the dotted line.

Peptides eluting earlier in the gradient are typically more hydrophilic, whereas peptides eluting later in the gradient are more hydrophobic. Peptide fractions were selected to cover a wide range of hydrophobicity and molecular weight as determined by MALDI-TOF mass spectral analysis (HP G2030A MALDI-TOF MS). Fractions selected for sequence analysis are numbered 1 through 15 in the figure.

The amino acid sequence of the peptides contained in the 15 fractions were determined. Unambiguous sequences for 20 peptides were determined and in each case identified the C-terminal residue as lysine. The results are summarized in the table.

Examples of these sequence analysis results are shown in Figure 2. The examples span the range of hydrophobicity and amino acid length (7 to 28 amino acids) commonly recovered from Lys-C proteolytic digests

These results demonstrate the high-efficiency sequence analysis of peptide sample derived from proteolytic digestions using the N-terminal sequence method “Routine 3.5” on the HP G1005A N-terminal Protein Sequencing System. The peptides analyzed span a broad range of peptide chain length, hydrophobicity, and sample amounts.

Peak number	Sequence identified[1]	Number residues
1	DSSLCK	6
	HQTVPQNTGGK	11
2	LHDRNTYEK	9
3	AVGNLRK	7
4	VPPRMDAK	8
5	CQSFRDHMK	9
6[2]	CQSFRDHMK	9
7	NPDPWAK	7
8	DSAHGFLK	8
9	DAGDVAFVK	9
	CDEWSVNSVGK	11
	DSGFQMNQLRGK	12
10	SVIPSDGPSVACVK	14
11	EFQLFSSPHGK	11
	LCMGSGLNLCEPNNK	15
12	INHCRFDEFFSEGCAPGSK	19
	DYELLCLDGTRK	12
13	EGYYGYTGAFCLVEK	15
14	MYLGYEYVTAIRNLREGTCPEAPTDECK	28
15	EDLIWELLNQAQEHFGK	17

1. Cysteines were identified through parallel digests in which the cysteines were converted to carboxyamido-methyl-CYS with iodoacetamide. Cam-CYS elutes between PTH-Gly and PTH-His (See AN 95-2).
2. MALDI-TOF mass analysis indicates that the Methionine in this peptide is oxidized.

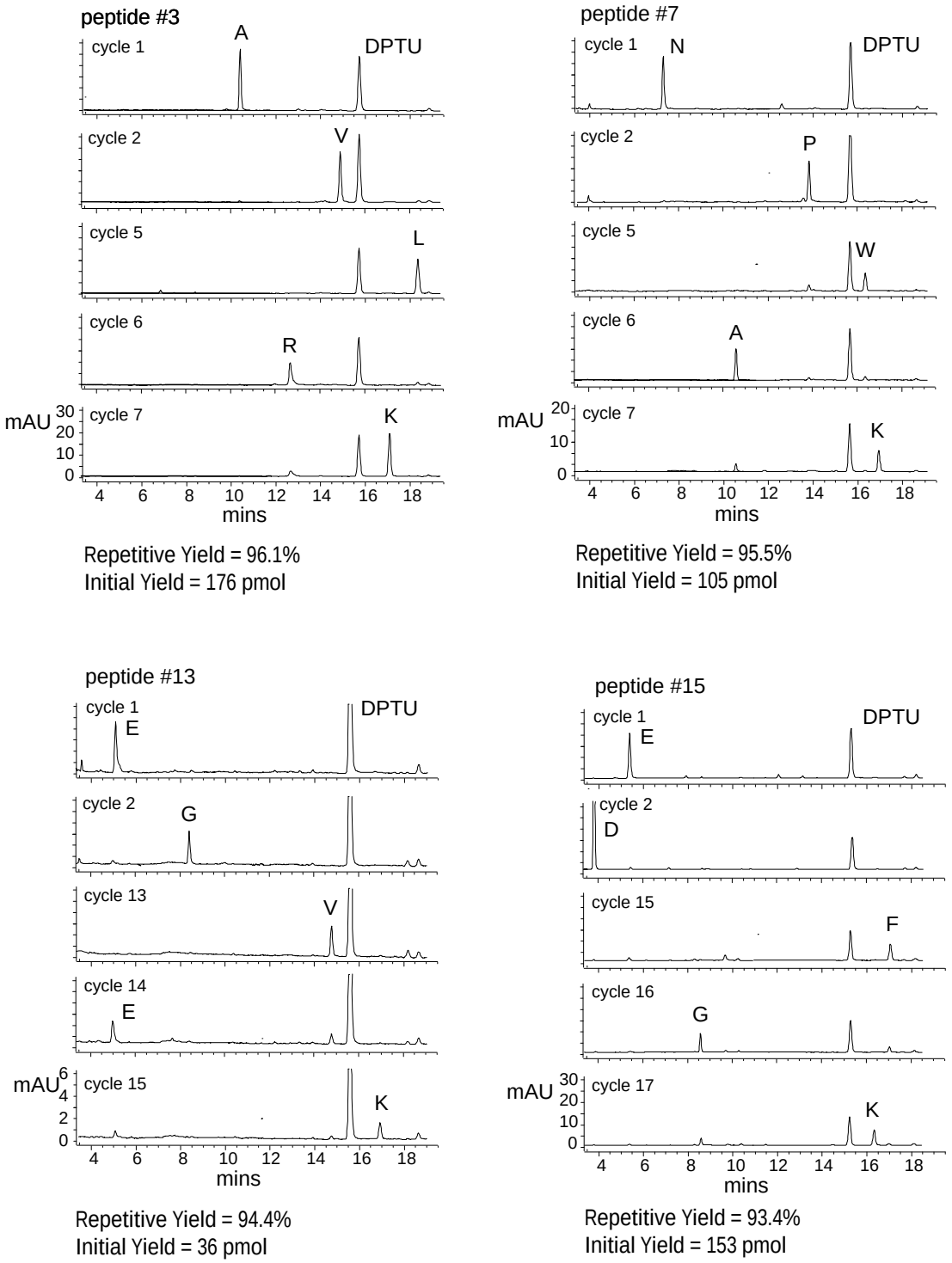

Fig 2. Results of sequence analysis of four peptides recovered from HPLC fractionation of the Lys-C digest of human apo-transferrin.

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Printed in the U.S.A. July 1996
(23) 5965-3465E